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(71) Applicant
Novo Industri A/S,
Novo Alle, DK-2880
Bagsvaerd, Denmark
(72) Inventors
Peder Øe Bargesgaard,
Georg Wilhelm Jensen,
Poul Holm
(74) Agent
Forrester, Ketley & Co.,
Forrester House, 52
Bounds Green Road,
London N11 2EY

(54) **Enzymatic Additive**

(57) A harshness reducing, enzymatic additive for a main wash detergent on the basis of a fungal cellulase, *i.e.* a cellulase produced by means of *Humicola insolens*. The enzymatic additive can be produced in high yields and has an extraordinarily high activity at alkaline pH values, whereby it is possible to mix the additive with a main wash detergent and perform the harshness reduction and the washing as a single operation.

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The information required by Rule 17(1)(a)(iii) of the Patents Rules 1978 was not contained in the application as filed, but was supplied later in accordance with Rule 17(2).

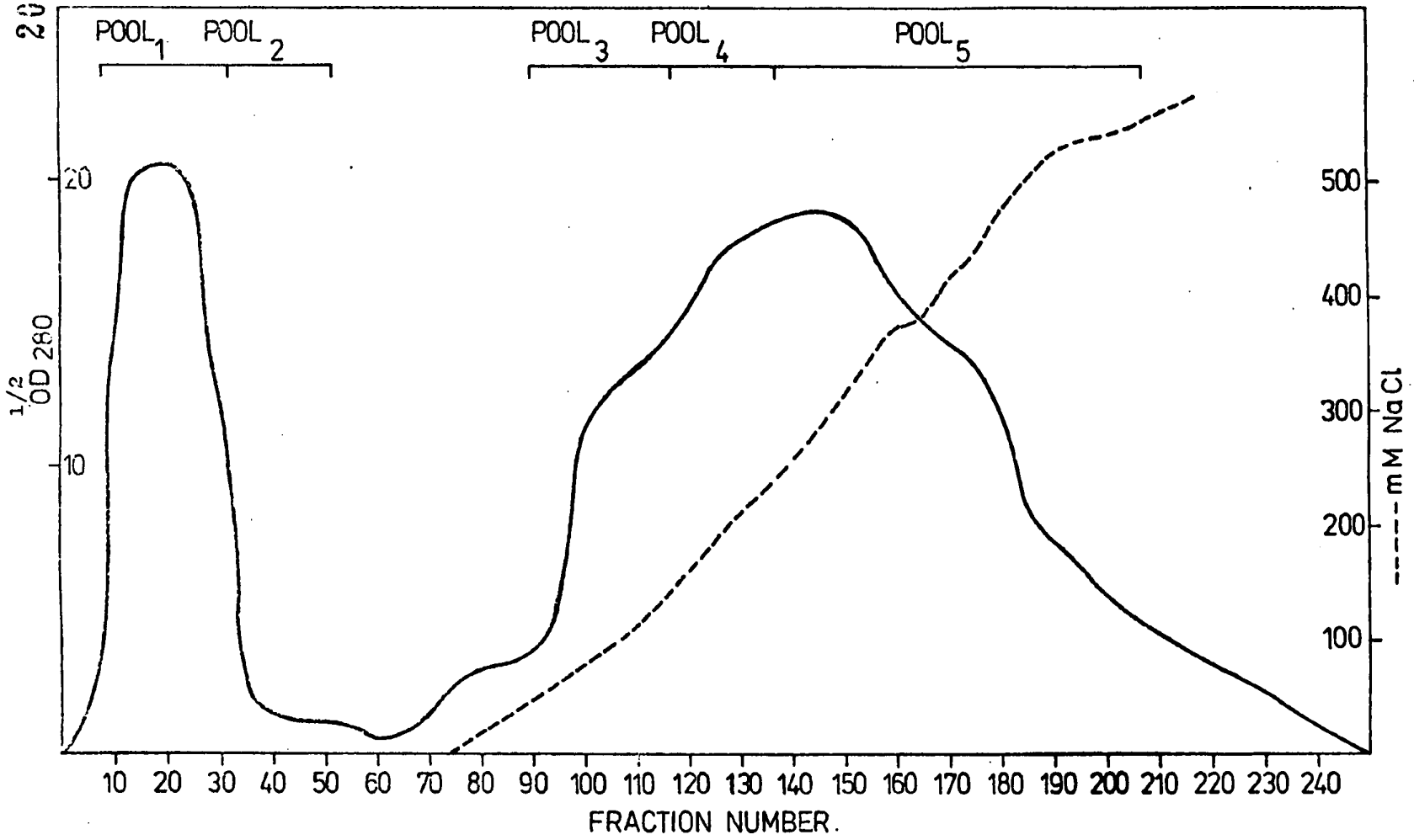

Fig.1

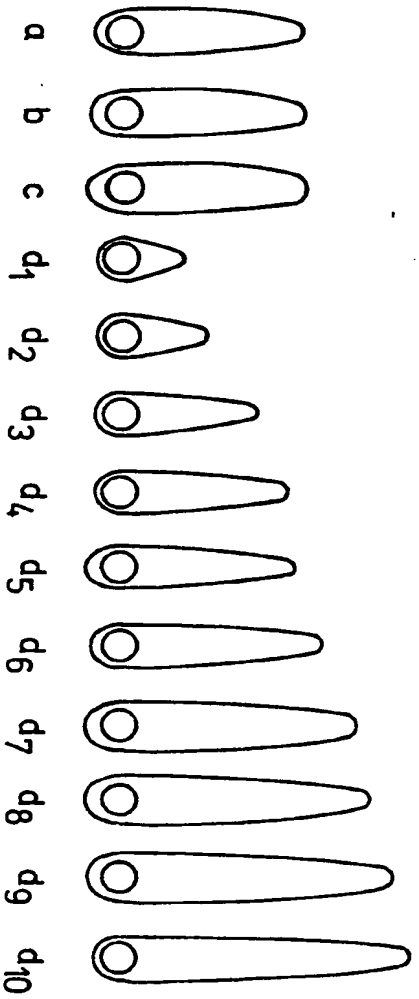

Fig.2

SPECIFICATION

Improvements In or Relating to an Enzymatic Additive

The present invention relates to improvements in or relating to a harshness reducing agent for a detergent composition, a detergent composition and a wash method.

It is known that washing of cotton-containing fabrics under normal conditions will generally cause a pronounced, unpleasant harshness. In current laundry practice, this harshness is normally reduced by treatment of the fabrics with a softening agent (rinse conditioner) containing a cationic detergent, in a step separate from the main wash. This treatment, however, has a serious drawback, that is that the harshness reducing effect is of a temporary nature. After the next wash the harshness will return, unless the fabric is treated again with a softening agent once more. In spite of the fact that large sums have been spent in order to find better ways of reducing harshness of the fabrics, the fact remains that the softening agents used in practice have to be added to the fabrics in a step separate from the main washing step in order to exert the full harshness reducing effect, and the harshness reducing effect is only of a temporary nature.

There are, however, various paper proposals that attempt to solve this problem. For example, in Specification No. 1,368,599 an alledged more efficient alternative for reducing harshness of cotton-containing fabrics is described, comprising treatment with a cellulase. It should be noted that the treatment is *always* conducted as a separate step, for example as a pre-wash or pre-soak, as will be explained in more detail hereinafter, but it is alledged in Specification No. 1,368,599 that cotton fabrics treated with a cellulase retain the softness for several washes. It should be noted, however, that the cellulase softening agent does not appear commercially, even though this technique was known as early as 1972 (when Belgian Patent Specification No. 773,280, an equivalent of Specification No. 1,368,599, was published). Thus, the teaching of Specification No. 1,368,599 (or Belgian Patent Specification 773,280) must be treated as a theoretical possibility rather than a realistic technical teaching, that the art has been willing to commercialise.

It should be noted that bacterial cellulases are usually rather expensive, because the yield thereof is normally very small, fungal cellulases are the most promising cellulases from a commercial point of view, because these can generally be produced in rather higher yields. For example, on page 2, lines 84—90 of the Queen's printers copy of Specification No. 1,368,599 it is stated: "Many types of cellulases derived from fungi have a pH-optimum of about 5. Above pH 7 their activity is normally greatly reduced, and therefore the celluiolytic enzymes derived from fungi should be used in the present invention in an acid medium". This means that the washing operation, which is normally carried out at an alkaline pH, cannot be carried out in the same step as the cellulase treatment. The cellulase treatment is carried out in a first step, as an acid pre-wash or pre-soak, and thereafter the main wash is carried out in a second step as an alkaline wash. As far as we are aware there has been no disclosure in the art of any cellulase intended to be used for the performance of a washing and softening in a single step in a practical washing procedure.

As will be appreciated, the necessity of carrying out the cellulase treatment at an acid pH before the main wash necessitates the use of an intermediate rinse between the acid cellulase treatment and the main wash, unless an excess of alkali for neutralising the acidity in the cellulase treated laundry is present in the detergent for the main wash or is added thereto.

Finally, if the cellulase treating liquid contains detergents, the cleaning ability of the detergent is normally rather low at the low pH-values in the cellulase treating liquid.

Thus an urgent need exists for a main wash detergent composition which contains a relatively cheap cellulase with high activity at the pH-values normally prevailing in main wash solutions.

However, the art does not point to any cheap cellulase which exhibits an acceptably high cellulase activity at the pH-values normally prevailing in main wash solutions in spite of the fact that a tremendous commercial gain might be expected if this need could be fulfilled.

Now, surprisingly, according to the invention it has been found that a certain fungal cellulase, i.e. the fungal cellulase producible from *Humicola insolens (Humicola grisea var. thermoidea),* has a high cellulase activity at the pH-values normally prevailing in main wash solutions contrary to what would be expected on the basis of common knowledge in the art, exemplified by the previously cited passage from the Queen's printers' copy of Specification No. 1,368,599.

Thus, according to the first aspect of the present invention there is provided a harshness reducing agent in detergent additive form for a detergent composition, an essential component of which is a fungal cellulase, wherein the fungal cullulase is producible by a strain of *Humicola insolens (Humicola grisea var. thermoidea),* and wherein the harshness reducing agent is a harshness reducing agent for a main wash d tergent composition.

Expressed in another way the invention is the use of the fungal cellulase producible from *Humicola insolens* as a harshness reducing ag nt for a main wash d tergent composition.

It is to be understood that the fungal cellulase producible from *Humicola Insolens* can be produced from regular strains of *Humicola Insolens* as well as from mutants and variants of *Humicola insolens*

By use of the harshness reducing agent for a main wash detergent composition in accordance

with the invention it has been possible for the first time to p rform a washing and harshness reducing process in a reasonably cheap way and in just one operation, that is in the main wash, without any pre-soaking or other pre-treatment.

It is described in Agric. Biol. Chem 44 (3), 481—487 (1980) and 44 (8), 1721—1728 (1980) that *Humicola insolens* is a cellulase producing thermophilic fungus. Also it is described that the optimum pH's of the cellulases produced by *Humicola insolens* are 5.3 and 5.0, respectively, and this cellulase, therefore, would seen to belong to the category of cellulases indicated above in connection with British Specification No. 1,368,599, consequently being completely unable to fulfill the purpose of the invention. Surprisingly, however, it has been found according to the invention that *Humicola insolens* produces a cellulase which fulfills the purpose of the invention.

Humicola Insolens falls within the group of thermophilic *Humicola* which comprises *Humicola insolens* and *Humicola var. thermoidea*, the taxonomic distinction between these two being very dubious.

The regular C_x cellulose activity is determined by virtue of the fact that cellulase hydrolyses carboxymethyl cellulose to reducing carbohydrates, the reducing ability of which is determined colorimetrically by means of the ferricyanide reaction, according to Hoffman, W. S., J. Biol. Chem. 120, 51 (1937).

The regular C_x cellulase activity is determined in relation to the substrate carboxymethylcellulose (CMC Hercules, 4 M6F).

The standard conditions are the following: pH 9.50; buffer 0.1 M tris(hydroxymethyl)aminomethan ("tris"); substrate 4 g CMC/litre of the above-indicated buffer solution; incubation temperature 50°C, and incubation time 20 minutes.

One regular C_x cellulase activity unit (for the sake of brevity in the following referred to as one regular C_x unit) is the amount of cellulase which, under the above-indicated standard conditions, forms an amount of reducing sugar equivalent to 1 μmol of glucose per minute.

In a preferred embodiment of the harshness reducing agent according to the invention the fungal cellulase is produced by means of the *Humicola* strain DSM 1800. It has been found that this strain produces an alkaline cellulase with a high C_x activity at alkaline pH values. The new *Humicola* strain has been identified at the Commonwealth Mycological Institute, Kew, England, as *Humicola insolens*. The new strain was deposited on 14th April 1980 at the German Collection of Microorganisms (DSM, Deutsche Sammlung von Mikroorganismen), Göttingen, Germany, under the DSM number 1800.

The following is a morphological and physiological description of the above new *Humicola* strain DSM 1800.

Turf: Short; mycelium at first white, later becoming dark grey with white patches; hyphae colourless, smooth, septate approximately 2.8 μ in diameter. Colourless drops of water sometimes present.

Reverse: Yellow becoming brown—dark grey with age.

Aleuriospores: Smooth; brown; produced singly, terminally on single or multicellular conidiophores; usually globose 12.6—16.8 μ or sometimes subglobose 7×11.2—14×16.8 μ. Apiculus present.

Chlamydospores: Produced singly or in chains; smooth; brown; 11.2—16.8 μ.

Phialocondia: Absent.

Temperature limits: No growth at 26°C; good growth from 37—50°C. Colonies 8 cm in diameter at 37°C for 5 days.

The above observations were made after 5 days at 37°C on YPSS agar with the composition indicated below.

Yeast extract Difco	4.0 g
K_2HPO_4	1.0 g
$MgSO_4 \cdot 7H_2O$	0.5 g
soluble starch	15.0 g
distilled water	1000 ml
agar	15.0 g

It has been found, surprisingly, that the cellulase product produced by means of the *Humicola insolens* strain DSM 1800, besides the softening effect, exhibits a strong dirt loosening and anti-redeposition effect.

In a preferred embodiment of the harshness reducing agent according to the invention the fungal cellulase is enriched in regard to the cellulase fraction, which does not attach itself to an anion exchanger at $6.5 \leqslant pH \leqslant 7.5$.

As will be shown in detail later (*vide* Example 6) it has been found that the cellulase fraction, which does not attach itself to an anion exchanger at $6.5 \leqslant pH \leqslant 7.5$ (f r the sake of brevity in the foll wing referred to as the AC_xI fraction) exhibits the most significant harshness reducing ability. It has also been found that the temperature stability of the AC_xI fraction was better than the temperature stability of the other fractions of the *Humicola insolens* cellulase and the temperature stability of the

acid fungal cellulas s described in the previously cited British patent No. 1,368,599.

In order to be able to ascertain the content of the AC_xI fraction the modified C_x (cellulase) activity may be determined in the following manner. A phosphate buffer A is made up of 26.52 g sodium dihydroxy phosphate dihydrate in 1000 ml of deionized water; thus the molarity of A is 0.17. 100 ml of A is mixed with approximately 800 ml of deionised water, pH is adjusted to 7.0 and the total volume of the buffer is adjusted to 1000 ml (buffer B), the molarity of which is 0.017. A column is loaded with 1 g of the anion exchanger DEAE-Sepharose CI-6B® and equilibrated with B. Then the enzyme is dissolved in B, and 25 ml of the thus prepared enzyme solution is passed through the above indicated, equilibrated column. The C_x activity of enzyme solution downstream of the column is determined in the manner indicated above for determination of the regular C_x cellulase activity. This C_x activity is termed the modified C_x activity and is smaller than the regular C_x activity in case some C_x activity is adsorbed to the anion exchanger under the condition specified.

In a preferred embodiment of the invention the fungal cellulase exhibits a ratio (C_x modified/$C_x$regular) of at least 0.6, preferably at least 0.8.

It has been found that such a harshness reducing agent is enriched in regard to the AC_xI fraction to such an extent that the harshness reducing ability is extraordinarily good.

In a preferred embodiment of the harshness reducing agent according to the invention, the fungal cellulase is provided as a non-dusting granulate. It is essential that the cellulase preparation has a low dust level. With a high dust level complications may arise both in factories where the cellulase preparation is mixed with detergents and during the later handling of the cellulase containing detergent. A granulate cellulase preparation can be prepared in a number of different ways, for example by means of a "Marumerizer" as described in Specifications Nos. 1,362,365 and 1,361,387 or by means of a granulating machine, as described in Aufbereitungs-Technik No. 3/1970, p. 147—153 and No. 5/1970, p. 262—278, or it can be a prilled granulate, as described in Belgian Patent Specification No. 760.135. In all cases, however, the granulate must have low dusting properties. The word "Marumerizer" is a trade mark.

In a preferred embodiment of the harshness reducing agent according to the invention, the cellulase granulate is coated with a whitening agent, preferably TiO_2, in combination with a dust binding agent. In this way, an attractive-looking cellulase granulate with a minimum of dusting ability is provided.

In another preferred embodiment of the harshness reducing agent according to the invention, the harshness reducing agent is a liquid in which the fungal cellulase is provided as a cellulase concentrate suspended in a non-ionic surfactant. The concentrate is produced on the basis of the fermentation broth. As previously mentioned, it is essential that the cellulase preparation has a low dust level. Because, in this embodiment the harshness reducing agent according to the invention is a liquid, the dust level thereof is zero, and the above-mentioned difficulties are thus avoided completely.

Advantageously, when the harshness reducing agent is a suspension, the non-ionic surfactant contains a thickening agent. Thereby, sedimentation of the cellulase can normally be avoided. As thickening agent fumed silica with an extremely small particle size may for example be used. If fumed silica is used as the thickening agent, the amount thereof is usually in the range of from 0.5 to 10 per cent (w/w), preferably in the range of from 1 to 5 per cent (w/w).

In a preferred embodiment of the harshness reducing agent according to the invention the harshness reducing agent is a liquid and the fungal cellulase is provided as a cellulase concentrate dissolved in an aqueous medium, preferably in the presence of a stabilizing agent. As stabilizing agent any agent can be used which stabilizes against sedimentation and against loss of enzymatic activity.

According to the second aspect of the present invention there is provided a main wash detergent composition, which comprises detergent ingredients and a harshness reducing amount of the harshness reducing agent of the first aspect of the present invention, whereby the pH of a solution of 1 g of the main wash detergent composition in 1 litre of water, with a hardness of 10° German before the addition of the main wash detergent composition, is in the range of from 7 to 10, preferably in the range of from 7.5 to 9.5.

It is intended that the expression "main wash detergent composition" covers a detergent composition which usually, but not necessarily, is used in a single wash and which is not preceded or followed by any other treatments of a laundry nature. The advantage associated with the main wash detergent composition according to the invention appears most clearly, when the main wash detergent composition according to the invention is used in a single wash which is not preceded or followed by any other treatments of a laundry nature.

The detergent ingredients in a main wash detergent composition in accordance with the pres nt invention are not critical, the only limitation being that these ingredients should be compatible with the fungal cellulase.

Thus, the detergent ingredients and the percentages thereof used may by the following, by way of example.

1) Surfactants, in particular anionic and non-ionic surfactants, in a total amount of from 1 to 100 per cent by weight, typically from 5 to 45 per cent. Typical anionic surfactants are linear alkyl aryl

sulphonates ("LAS") and α-olefin sulphonates ("AOS"). Typical non-ionic surfactants are alkyl phenyl ethoxylates and fatty alcohol ethoxylates.

2) Builders, in particular alkaline builders, and water hardness reducers, in a total amount of from 5 to 80 per cent by weight, typically from 25 to 75 per cent. Typical builders are sodium tripolyphosphate ("STPP"), sodium aluminium silicates (zeolites), sodium silicates and sodium carbonates.

3) Bleaching agents, in particular peroxides in a total amount of from 0 to 40 per cent by weight, typically 0 per cent or from 15 to 30 per cent. Typical bleaching agents are sodium perborate and sodium percabonate.

4) Other ingredients, predominantly enzymes, optical brighteners, perfumes, dyes, foam modifiers, stabilisers, antiredeposition agents, preferably not CMC, which to a certain extent will be decomposed by the cellulase, in a total amount of from 0 to 10 per cent by weight, typically from 0.1 to 5 per cent.

5) Fillers, in particular sodium sulphate, and water, in such amount as to bring the total of the detergent formulation to 100 percent.

In the brochure "Novo Enzymes" for the detergent industry (B 157a-GB 2000, Feb. 1977), other general examples of detergent formulae, which could be used together with the cellulase preparation, are listed, namely compositions characterised by either specific compounds or categories of compounds and corresponding percentages thereof or corresponding ranges for the percentages thereof.

On page 5 of the Danish Patent Application No. 5691/78, filed on 12th December 1978, some specific detergent compositions, which could be used together with the cellulase preparation, are listed.

In a preferred embodiment of the main wash detergent composition in accordance with the present invention, the harshness reducing agent is present in an amount corresponding to from 2.5 to 100 regular C_x units/g of main wash detergent, or from 1.5 to 60 modified C_x units/g of main wash detergent, preferably from 5 to 50 regular C_x units/g of main wash detergent, or from 3 to 30 modified C_x units/g of main wash detergent. In this way a satisfactory harshness reducing effect can be obtained, and also an excess of cellulase (which would make the main wash detergent composition uneconomical) can be avoided.

In a preferred embodiment of the main wash detergent composition in accordance with the present invention, the main wash detergent also contains a bacterial proteinase, preferably a proteinase produced by means of *Bacillus lichenformis*, in particular ALCALASE. Surprisingly, it has been found that the detergent additives cellulase and proteinase are compatible, and that they both exert a satisfactory effect at the pH values during the washing procedure, even though it could be thought that the proteolytic enzyme would digest the cellulase. This combined detergent additive exerts both a better cleaning effect and a better softening effect when added to the detergent. The word "ALCALASE" is a Trade Mark for a Novo enzyme preparation commercially available. If ALCALASE and a granulate of cellulase is used, the mixed enzymatic additive can be prepared either by mixing a previously prepared granulate of ALCALASE with a previously prepared granulate of cellulase or by mixing a concentrate of ALCALASE with a concentrate of cellulase and then introducing this mixture into a granulating device, together with the usual granulating aids.

In a preferred embodiment of the main wash detergent composition in accordance with the present invention, perborate is one of the detergent ingredients. Under certain conditions, perborate enhances the washing efficiency and it has surprisingly been found that perborate and the cellulase in the main wash detergent composition according to the invention are compatible.

According to the third aspect of the present invention there is provided a main wash method, in which a main wash detergent composition in accordance with the second aspect of the present invention is used as the detergent.

In a preferred embodiment of the main wash method in accordance with the present invention, the fungal cellulase is used in a concentration in the washing solution corresponding to from 10 to 100 regular C_x units/litre washing solution, or from 6 to 60 modified C_x units/litre of washing solution, preferably from 20 to 60 regular C_x units/litre of washing solution, or from 12 to 30 modified C_x units/litre of washing solution.

In a preferred embodiment of the invention the pH in the washing solution is between 7 and 10, preferably between 7,5 and 9,5. Hereby the cellulase is able to exert its full activity.

In a preferred embodiment of the main washing method in accordance with the present invention a significant part of the main wash is performed at a temperature below 70°C, preferably below 60°C.

The following examples illustrate further the fermentation, by means of which the harshness reducing agent in accordance with the invention is produced (examples 1, 6 and 8), and main wash detergent compositions in accordance with the invention and main wash methods in accordance with th invention (exampl s 2—8).

Example 1

The strain DSM 1800 was cultivated at 37°C on an agar substrat with the composition:

Yeast extract Difco	4 g
K_2HPO_4	1 g
$MgSO_4.7H_2O$	0.5 g
Glucose	15 g
Distilled water	1000 g
Agar	15 g

A primary group of 500 ml shaking flasks was prepared; these shaking flasks contained 100 ml of a substrate with the composition:

Corn steep liquor	2.4 per cent
Glucose	2.4 per cent
$CaCO_3$	0.5 per cent
Soy oil	0.5 per cent

Before the addition of $CaCO_3$, the pH value was adjusted to 5.5 with 4N NaOH, and sterilisation was performed at 121°C for 20 minutes.

After growth for 7 days on the agar slant the spores were transferred to the shaking flasks in the primary group of 500 ml shaking flasks.

A secondary group of 500 ml shaking flasks was prepared; these shaking flasks each contain 100 ml of a substrate with the composition:

NH_4NO_3	0.25 per cent
KH_2PO_4	0.56 per cent
K_2HPO_4	0.44 per cent
$MgSO_4.7H_2O$	0.075 per cent
Cellulose	2.0 per cent
$CaCO_3$	0.25 per cent
Pluronic	0.01 per cent

Before the addition of $CaCO_3$, the pH value was adjusted to 6.8—7.0 by means of 4N NaOH, and sterilisation was performed by treatment in an autoclave at 121°C for 20 minutes.

After growth for 2x24 hours at 37°C in the primary group of shaking flasks, inoculation with 6 per cent from the primary group of shaking flasks to the secondary group of shaking flasks was performed.

After fermentation in the secondary group of shaking flasks for 140 hours at 37°C the cellulase activity was 4.2 regular C_x units/ml culture broth. Then the culture broth was purified in the following manner: The culture broth was filtered on diatomaceous earth (Hyflo-super-cell), the filtrate was concentrated by ultrafiltration, and the retentate was freeze dried. The freeze dried powder (A) showed a C_x activity of 745 regular C_x units/g.

Two experiments were carried out in exactly the same manner as described above. After fermentation in the secondary group of shaking flasks for 140 hours the regular C_x cellulase activity in these two experiments was 2.2 and 6.0 C_x units/ml culture broth, respectively. Also, the freeze dried powder in these two experiments showed a regular C_x activity of 558 (powder B) and 518 C_x units/g (powder C).

Example 2

In this example the cellulase preparation powder A from example 1 was used as the harshness reducing agent.

The washing experiment was carried out as a one-step wash with a fully automatic MIELE drum washing machine in accordance with the washing programme called "Kogevask 95°C" indicated in the T-52001 brochure describing the MIELE washing machine type 421 S. The word "MIELE" is a Trade Mark. The programme called "Kogevask 95°C" has the following approximate temperature-time profile:

time (min.)	0	6	12	18	24	30	36	42	45	
Temp. (°C)	16	27	43	61	76	85	85	82		flushing with cold water

The washing solution had the following composition: approximately 16 litres of water with hardness of approximately 20° dH containing 5 g/litre of a conventional, commercial powder detergent with the following main ingredients (approximate dry solids composition w/w):

21% of surfactants (7% of LAS, 11% of soap, and 3% of nonylphenyl-ethoxylate)
39% of builders (32% of STPP, 4% of sodium silicate, and 3% of sodium carbonate)
26% of sodium perborate
13% of sodium sulphate

To this washing solution was added th proteolytic enzyme preparation of ALCALASE in a concentration corresponding to 0.06 Anson units/litre of solution. The proteolytic activity of th enzyme preparation was determined according to the modified Anson method, described in Novo Analytical Method No. AF 4.3/5-GB (the original Anson method is described in J. Gen. Physiol., 22, 79—89 (1939)).

To this washing solution was added either nothing or the cellulase preparation from Example 1 in a concentration corresponding to approximately 40 regular C_x units/litre of solution.

The test materials used for each of these two parallel experiments were:

1) 12 pieces of white Terry cloth of cotton with dimensions 42 cm×60 cm, and
2) 12 pieces of white cotton interlock with the same dimensions.

Furthermore, clean cotton laundry was supplied with the two kinds of test material to provide a total of 3 kg of laundry. The test material was washed 20 times before use with a perborate-containing, conventional detergent using a high temperature programme.

After these 20 repeated washes, simulating washing of the test material in practice, the test material was ready for the washing experiment with or without cellulase.

The test material was washed 6 times with intermediate drying on a line over night. After the final wash, all test pieces (12×2×3) were conditioned on a line in the same room.

The evaluation was performed by a panel consisting of 20 persons. Without knowledge of the identity of the different test pieces, each of the first 10 persons in the panel was asked to make 6 evaluations (that is, 3 for the white Terry cloth and 3 for the white cotton interlock) on the basis of which it was possible to select the correct statement among the following statements:

1) The cloth treated with cellulase is softer than the cloth treated without cellulase,
2) The cloth treated with cellulase has the same degree of softness as the cloth treated without cellulase, and
3) The cloth treated with cellulase is harder than the cloth treated without cellulase.

3 pieces of test material were selected from the 12 pieces of white Terry cloth of cotton treated with cellulase, and 3 pieces of the test material were selected from the 12 pieces of white Terry cloth of cotton treated without cellulase. These 6 pieces were arranged into 3 pairs, and each of the first 10 members of the panel was asked to evaluate all 3 pairs separately. Similar evaluations were carried out with the white cotton interlock. The remaining 10 persons in the panel were then asked to evaluate the remaining test material in the same way.

Referring only to the white Terry cloth, each person in the panel made 3 evaluations, corresponding to a total from the entire panel of 60 evaluations for this test material.

The white cotton interlock was evaluated in the same manner, the total number of evaluations also being 60 in this case.

The result of the above described evaluation appears from the following Table 1. Table 1 also shows the pH of the detergent solution at the start of the wash, after 10 minutes and after 20 minutes (t=0, t=10, and t=20, respectively).

Example 3

Example 2 was repeated. The results appear in Table 1.

Example 4

In this example the cellulase preparation powder B from example 1 was used as the harshness reducing agent. Example 2 was repeated, but with the following changes: The washing machine was filled with test material as in example 2, and then dirty cotton laundry was supplied to provide a total of 3 kg of laundry. The dirty laundry was cotton underwear from a military camp. The results appear in Table 1.

Example 5

In this example the cellulase preparation C from example 1 was used as the harshness reducing agent. The experiment described in example 2 was repeated, but with the following changes: The washing programme called "Finvask 60°C" in the T-52001 brochure was used. This programme has the following approximate temperature/time profile:

time (min)	0	6	12	18	24	30	36	42	
temp. (°C)	16	24	35	47	57	55	54		flushing with cold water

The washing solution had a volume of approximately 26 litres. The pH of the washing solution was adjusted slightly with citric acid. The results appear in Table 1.

Table 1

Example	*Test material*	*Evaluation*
Ex. 2 pH at t=0: 10.0 t=10: 9.8 t=20: 9.7	terry cloth	73%: softer with cellulase 2%: no difference 25%: harder with cellulase
	cotton interlock	77%: softer with cellulase 8%: no difference 15%: harder with cellulase
Ex. 3 pH at t=0: 9.9 t=10: 9.8 t=20: 9.6	terry cloth	68%: softer with cellulase 5%: no difference 27%: harder with cellulase
	cotton interlock	78%: softer with cellulase 2%: no difference 20%: harder with cellulase
Ex. 4 pH at t= 0: 9.8 t=10: 9.7 t=20: 9.6	terry cloth	84%: softer with cellulase 3%: no difference 13%: harder with cellulase
	cotton interlock	82%: softer with cellulase 5%: no difference 13%: harder with cellulase
Ex. 5 pH at t= 0: 8.9 t=10: 8.8 t=30: 8.9	terry cloth	93%: softer with cellulase 0%: no difference 7%: harder with cellulase
	cotton interlock	93%: softer with cellulase 2%: no difference 3%: harder with cellulase

Example 6

The strain DSM 1800 was cultivated at 37°C on an agar substrate with the same composition as the agar substrate in Example 1.

300 litres of an inoculation or pre-fermentation substrate with the composition:

Corn steep liquor	2.4 per cent
Glucose	2.4 per cent
$CaCO_3$	0.5 per cent
Soy oil	0.4 per cent

(before the addition of $CaCO_3$, the pH was adjusted to 5.5 by means of 4N NaOH) was sterilised at 121°C for 45 min.

After growth for 14 days on the agar substrate the spores were transferred to the sterilised 300 l pre-fermentation substrate. Sterile air was passed through the thus-prepared pre-fermentation broth for 49 hours at 37°C under a pressure of 0.5 atmospheres and at a rate of 300 litres per minute.

350 litres of a main fermentation substrate having the composition:

Corn steep liquor	10 per cent
Cellulose	3 per cent
$CaCO_3$	0.25 per cent
NH_4NO_3	0.25 per cent
KH_2PO_4	0.56 per cent
K_2HPO_4	0.44 per cent
$MgSO_4$	0.08 per cent
Pluronic	0.014 per cent

(before the addition of $CaCO_3$, the pH was adjusted to 6.8 to 7.0 by means of 4N NaOH) was sterilised by boiling at 123°C for 60 min. 35 litres of th pre-fermentation broth was transferred to the thus-sterilised main fermentation substrate. Sterile air was passed through the thus-prepared main

fermentation broth under a pressure of 0.5 atmospheres and at a rate of 300 litres per min. pH was kept below 7.0 with addition of KH_2PO_4. Th fermentation was carried out at 37°C for 136 hours. At the conclusion of the fermentation the activity was 29 regular C_x units/ml.

The culture broth was purified in the following manner:

The culture broth was filtered on diatomaceous earth (Hyflo-super-cell). The filtrate was concentrated by ultrafiltration, and the retentate (which for the sake of brevity in the following will be referred to as retentate D) was freeze dried.

The freeze dried powder D showed a regular C_x activity of 1316 C_x units/g, and a modified C_x activity of 953 C_x units/g.

Two more fermentations with subsequent ultrafiltrations were carried out in the same way as indicated above. The corresponding retentates are referred to as retentate E and F. Retentate F was freeze dried, and the freeze dried powder dried powder F showed a regular C_x activity of 743 units/g and a modified C_x activity of 580 units/g.

The freeze dried powder D was separated in different components by anionic exchange chromatography in the following manner: 100 g thereof was dissolved in 2000 ml of distilled water at 4°C. The pH was adjusted to 7.5 with 1 M "tris". The solution was then applied to a 4000 ml ion exchange column with 100 g of DEAE-Sephadex A-50. Figure 1 shows the absorbance OD_{280} at 280 nm of the fractions released from the column. The proteins bound to the column were eluted by a NaCl gradient; the NaCl concentration was measured and plotted on figure 1 together with the absorbance of the fractions.

The protein containing fractions were pooled as indicated with the brackets on figure 1 and the pooled fractions numbered 1 to 5. Pools 1 to 5 were then freeze dried separately.

The freeze dried pools were characterised by the amount of protein, the composition of proteins and the regular C_x activity, *vide* table 2. The isoelectric point of the proteins was determined with a LKB multiphoer apparatus.

Table 2

	Protein, g	*Total regular C_x units*	*pI range*	*Yield protein, %*	*Yield C_x %*
Starting material	53.0	131600	3.5—9.0		
Pool 1[x)]	8.6	47400	6—9.0	16	36
Pool 2	0.9	1000	4—8	2	1
Pool 3	4.8	9500	4—5.5	9	7
Pool 4	7.1	11000	3,5—5.5	13	8
Pool 5	17.7	18600	3,5—5	33	14
			Total	73	66

x) (=AC_xI fraction)

Pool 1 contains all the alkaline components, i.e. the proteins which are not bound to the anionic exchange resin at $6.5 \leqslant pH \leqslant 7.5$. Thus, the total amount of modified C_x cellulase activity is present in pool 1, which also contains all the cellulolytic activities with pI above 6.0.

In order to evaluate which of the five pools contained the fraction with the highest softening effect the following experiments were carried out.

Cotton terry cloth was prewashed 20 times as described in Example 2. Following this prewash, 10 cm×10 cm (approx. 4 gram) swatches were cut out and marked.

The cellulase treatment was now carried out in a Terg-O-Tometer laboratory washing machine under the following conditions:

Detergent and dosage: as in Example 2, except for cellulase dosage

Initial pH	approx. 9.5
Temperature:	50°C
Time:	30 min.
Water hardness:	20° dH
Volume per beaker:	1200 ml
No. of swatches per beaker:	10
Cellulase dosage:	see table below, in all cases 100 mg of the freeze dried pools/litre

Dosage:	*Pool 1*	*Pool 2*	*Pool 3*	*Pool 4*	*Pool 5*
mg protein/l	31	13	28	34	52
regular C_x units/l	170	15	56	52	55
modified C_x units/l	170	0	0	0	0

The evaluation was carried out by a panel consisting of 10 persons, who were given different sets of swatches, each consisting of three swatches, and requested to arrange the swatches within each set according to softness. Each set consisted of two swatches, which were washed with one of the pools, and one swatch served as a control. 9 persons of the 10 persons in the panel found that the swatches washed with pool 1 were softer than the one with no pool added. All the other pools did not significantly soften the swatches.

From the above it may be concluded that pool 1 contains C_x activity which has a softening effect. The C_x activity in the pools 3—5 exhibits no significant softening effect at dosage levels, which normally provide a softening effect.

Example 7

Retentate E from Example 6 was spraydried, whereby a crude concentrate was formed.

This crude concentrate was forced through a screen with a hole diameter of 0.5 mm, thus obtaining a mean diameter of 17.4 μm. The cellulase activity of the concentrate in this condition was 650 regular C_x units/g.

A mixture consisting of

The above cellulase concentrate	2.5 kg
Cellululose powder (Arbocel BC 200)	1.0 kg
TiO_2	0.2 kg
NaCl (particle size <0.2 mm)	6.1 kg

was mixed in a mixer (Lödige FM 50/IMZ) at a rotational speed of 120 r/m and a knife speed of 3000 r/m. To the thus produced mixture was added 1 kg of a 20% aqueous solution of polyvinyl pyrrolidon (PVP K 30), whereby the above indicated rotational speed and knife speed was maintained. The addition of the aqueous solution was carried out by means of a pressure nozzle and lasted for 1 minute. This wet mixture then was further treated for 3 minutes in the mixer at a mixing speed of 200 r/m and a knife speed of 3000 r/m. Twice a further amount of water was added, viz, 200 g and 100 g of water, respectively, both times followed by treatment in the mixer of a duration of 3 minutes. It was observed that the temperature in the mass rose from 25°C to 30°C during granulation. The moist granulate was dried in a fluid bed to a water content of 1.6%. The words "Lödige" and "Arbocel" are trade marks.

Regular C_x activity of dry granulate:

Calculated	164 regular C_x units/g
Found	136 regular C_x units/g
Activity loss	17%.

This product was a particulate product with lens shaped particles. For the sake of brevity it will be referred to as granulate E.

Granulate E exhibited the following grain size distribution:

Particle size, μm	*% of particles*
>1180	15
>1000	28
> 850	43
> 707	62
> 600	76
> 500	87
> 420	94
> 300	1.8

Mean diameter=790 μm.

Granulat E was coated in the following manner. 4,0 kg of granulate E was dumped into a mix r (Lödige, type M 20E). The mixture was heated to 65°C, and then 160 g of melted polyethylene glycol 4000 (molecular weight 4000) was added; this mixture was mixed for 1 minute. Subsequently the granulate was powd red with 360 g of TiO_2 and 90 g of magnesium silicat . After a mixing time of 5

minutes further 40 g of polyethylene 4000 was poured onto th mixture, and the mixing was continued for 1 minute. The charge was left for cooling. 1 kg of the thus produced coated granulate was dust blown for 1.5 minutes at 65°C on a fluid bed.

The activity of the granulate appears from the following analysis:

	Coated granulate	*Coated and dust blown granulate*
Activity, Regular C_x units/g	137	138

The above indicated coated granulate, the coated and dust blown granulate, and granulate E are typical embodiments of a harshness reducing agent in detergent additive form.

A washing experiment with granulate E was carried out as in Example 6, with the following change:

Cellulase dosage:

333, 667, 1000, 1333, and 1667 mg/liter.

The evaluation was carried out as in Example 6, with the following changes:

Each set of swatches consisted of 2 swatches, namely one swatch washed with detergent solution containing inactivated cellulase, and one swatch washed with detergent solution containing active cellulase. The inactivated cellulase was obtained by preparing a stock solution with 10 g granulate E dissolved in water to 250 ml and heating this solution to 90°C and maintaining this temperature for 10—15 minutes. A total of 5 sets were evaluated and the results of the evaluations are shown in Table 3 below:

Table 3
Softness Evaluation (%)

Set No.	*Cellulase dosage (mg/liter)*	*Cellulase*	*inact. cellulase*
		Evaluation: swatch treated as indicated is softest says:	
1	333	60%	40%
2	667	70%	30%
3	1000	70%	30%
4	1333	70%	30%
5	1667	90%	10%

Example 8

In this example different strains of *Humicola insolens* were compared, i.e. CBS 14764 (identical to ATCC 22082), DSM 2069 and DSM 2068 DSM 2068 and DSM 2069 were deposited with Deutche samm lung von mikroorganismen on 30 March, 1981. These strains were cultivated as described in Ex. 1, with the exception that the substrate for the secondary group of shaking flasks besides the constituents indicated in ex. 1 contained 14% corn steep liquor. Freeze dried powders were produced from the fermentation broths as indicated in ex. 1. The freeze dried powders were desalted, redissolved and desalted on a Sephadex G 25 column and freeze dried again. These freeze dried powders exhibited 1096, 480 and 690 regular C_x units/g, respectively. Also a portion of the freeze dried powder F from Example 6 (DSM 1800) was used in this example.

The freeze dried pool 1 from example 6 was purified further by cation exchange on CM-Sepharose CL 6B. An amount of this freeze dried pool 1 corresponding to 1.7 g protein was dissolved in a citric acid buffer at pH 5.0 and applied to a 1000 ml column with the cation exchanger, to which the protein was attached. It was eluated with a NaCl gradient, and at a NaCl concentration of 250 mM the pure CMC active AC_xI protein was eluated. The molecular weight of this protein is found to be 80,000 by means of SDS gel electrophoresis. Also, this protein was used for preparation of antiserum (rabbits).

This antiserum was now used for quantification of the content of the AC_xI protein by means of immunological techniques, i.e. rocket immunoelectrophoresis, as described by B. Weeke in Scandinavian I. Immun., 2 Suppl. 1, page 37 (1973).

The rocket immunoelectrophoresis pattern is shown on fig. 2. The electrophoresis was performed over night in a tris maleate buffer of pH 7 and with a voltage gradient of 1.0 Volt/cm.

In fig. 2 a corresp nds to th DSM 2069 product (3 mg/ml), b corresponds to the CBS 14764 product (2 mg/ml, c corresponds to the DSM 2068 product (3 mg/ml and d_n corresponds to the DSM 1800 product (n mg/ml).

The above indicated c llulase preparations originating from DSM 1800, CBS 14764, DSM 2068 and DSM 2069 were used in the washing experiment d scribed below:

Cotton terry cloth was prewashed 20 times as described in Example 2. Following this prewash, 10 cm x 10 cm (approx. 4 gram) swatches were cut out and marked.

The cellulase treatment was now carried out in a Terg-O-Tometer laboratory washing machine under the following conditions:

Detergent and dosage: as in Example 2, except for cellulase dosage

Initial pH:	approx. 9.5
Temperature:	50°C
Time:	30 min.
Water hardness:	20° dH
Volume per beaker:	1200 ml
No. of swatches per beaker:	10
Cellulase dosage:	see table below

Cellulase dosage (mg/litre)	*none*	*DSM 1800*	*CBS 14764*	*DSM 2068*	*DSM 2069*
	0	102	39	96	105
regular C_x units/litre		76	43	66	50

The cellulase preparation originating from DSM 1800 was added in a dosage of 76 regular C_x units/litre, and the other cellulase preparations were added in a dosage to generate the same AC_xI protein concentration (determined immunologically as described previously in this example) in the wash solution as the DSM 1800 preparation. The cellulase treatments were repeated 6 times. After each treatment the swatches were thoroughly flushed in tap water and air dried on a line overnight.

The evaluation was carried out by a panel, consisting of 10 persons, who were given 4 different sets of swatches, each consisting of 3 swatches, and requested to arrange the swatches within each set according to softness. The softest swatch in a set was given a "1", the next a "2", and so on. The composition of the sets evaluated and the results of the evaluations, expressed in per cent (the percentage of the panel which places a certain swatch in a certain softness category), are shown in Table 4 below:

Table 4
Softness Evaluation (%)

Set No.	*Cellulase*	*Softness category*		
		"1" softest	*"2"*	*"3"*
1	none	0	10	90
	powder C (ex.6)	80	20	0
	powder from CBS 14764	20	70	10
2	none	0	30	70
	powder C (ex.6)	70	30	0
	powder from DSM 2068	30	40	30
3	none	0	20	80
	powder C (ex.6)	30	50	20
	powder from DSM 2069	70	30	0

Claims

1. A harshness reducing agent in detergent additive form for a detergent composition, an essential component of which is a fungal cellulase, wherein the fungal cellulase is producible by a strain of *Humicola insolens (Humicola grisea var. thermoidea),* and wherein the harshness reducing agent is a harshness reducing agent for a main wash d tergent composition.

2. A harshness reducing agent according to Claim 1, wherein the strain of *Humicola insolens* is that deposited under DSM 1800.

3. A harshness reducing agent according to Claim 1—2, wherein the fungal cellulase is enriched in regard to th cellulase fraction which does not attach itself to an anion exchanger at $6.5 \leqslant pH \leqslant 7.5$.

4. A harshness reducing agent according to Claim 3, wherein the fungal cellulase exhibits a ratio (C_x modified/C_x regular) of at least 0.6, preferably at least 0.8.

5. A harshness reducing agent according to Claim 1—4, wh rein the harshness reducing agent is a non-dusting granulate.

6. A harshness reducing agent according to Claim 5, wherein the non-dusting granulate is coated with a whitening agent in combination with a dust binding agent.

7. A harshness reducing agent according to Claim 6, wherein the whitening agent is TiO_2.

8. A harshness reducing agent according to Claim 1—4, wherein the harshness reducing agent is a liquid and wherein the fungal cellulase is provided as a cellulase concentrate suspended in a non-ionic surfactant.

9. A harshness reducing agent according to Claim 8, which additionally contains a thickening agent.

10. A harshness reducing agent according to claim 1—4, wherein the harshness reducing agent is a liquid and wherein the fungal cellulase is provided as a cellulase concentrate dissolved in an aqueous medium, preferably in the presence of a stabilizing agent.

11. A main wash detergent composition, which comprises detergent ingredients and a harshness reducing amount of the harshness reducing agent according to any one of Claims 1—10, whereby the pH in a solution of 1 g of the main wash detergent composition in 1 litre of water with a hardness of 10° German before the addition of the main wash detergent composition is in the range of from 7 to 10, preferably from 7.5 to 9.5.

12. A main wash detergent composition according to Claim 11, wherein the harshness reducing agent is present in an amount corresponding to from 2.5 to 100 regular C_x units/g of main wash detergent composition or from 1.5 to 60 modified C_x units/g of main wash detergent composition.

13. A main wash detergent composition according to Claim 12, wherein the harshness reducing agent is present in an amount corresponding to from 5 to 50 regular C_x units/g of main wash detergent composition or from 3 to 30 modified C_x units/g of main wash detergent composition.

14. A main wash detergent composition according to Claim 11,12, or 13 which also contains a bacterial proteinase.

15. A main wash detergent composition according to Claim 14, wherein the bacterial proteinase is a proteinase produced by means of *Bacillus lichenlformis*.

16. A main wash detergent composition according to any one of Claims 11—15, wherein one of the detergent ingredients is a perborate.

17. A main wash method in which a main wash detergent in accordance with any one of Claims 11—16 is used as the detergent.

18. A main wash method according to Claim 17, wherein the fungal cellulase is used in a concentration in the washing solution corresponding to from 10 to 100 regular C_x units/litre of washing solution or from 6 to 60 modified C_x units/litre of washing solution.

19. A main wash method according to claim 18, wherein the fungal cellulase is used in a concentration in the washing solution corresponding to from 20 to 50 regular C_x units/litre of washing solution or from 12 to 30 modified C_x units/litre of washing solution.

20. A main wash method according to claim 17—19, wherein the pH in the washing solution is between 7 and 10, preferably between 7.5 and 9.5.

21. A main wash method according to claim 17—20, wherein the main part of the main wash is performed at a temperature below 70°C.

22. A main wash method according to claim 21, wherein the main part of the main wash is performed at a temperature below 60°C.

23. A harshness reducing agent for a main wash detergent composition, in accordance with claim 1 and substantially as described in anyone of the foregoing examples.

24. A main wash detergent composition in accordance with claim 11 and substantially as described in the foregoing examples.

25. A main wash method according to claim 17 and substantially as described in the foregoing examples.

26. Any novel feature or combination of features described herein.

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